2nd PROGRESS REPORT

PROJECT TITLE: Functional Characterization of Cystinosin-LKG
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A preliminary study performed *in silico* on the last 39 amino acids of cystinosin-LKG carboxy terminal tail has identified potential phosphorylation sites in serine (Ser) and threonine amino acid residues. In particular, we have initially selected the Ser in position 396 and 397 of protein "SSLK" motif that are strongly preserved in evolution chain from mouse to homo sapiens. These amino acids have been mutated in alanine (Ala) or aspartic acid (Asp) that blocks and mimics phosphorylation, respectively.
Preliminary results have shown that Ala substitution in both positions induced an increase of cystinosin-LKG expression on plasma membrane whereas Asp mutation in 397 position induced a decrease of the protein trafficking toward plasma membrane. No data are available for Ser 396 to Asp substitution. We are generating double mutants that substitute Ser to Ala or Asp in position 396-397 of cystinosin-LKG protein for studying an potential additional effect dues to phosphorylation.
To study the cystinosin-LKG phosphorylation sites and binding interactions, we have performed analysis in tandem mass spectrometry (MS/MS) of immuno-precipitation products. Preliminary data show vimentin, an intermediate filament protein in the immuno-precipitate of cystinosin-LKG. These data are supported by previous immuno-fluorescence images that showed co-localization of cystinosin-LKG with kinesin in MDCK cells. The interaction of cystinosin-LKG with cytoskeleton proteins suggests trafficking of vesicles carrying this protein in different cellular districts.

To deepen the study cystinosin-LKG cellular localization we have expressed this isoform conjugated with fluorescence protein (DsRED) in MDCK cells that polarize when they are cultured on polycarbonate substrate. Analysis was performed by confocal laser scanning microscopy. The cystinosin-LKG that located on plasma membrane was prevalently concentrated on basolateral face.

Overall, these data support the concept that this isoform has a specific role in cells. Moreover, initial data that have been presented by Dr Goodyer in collaboration with our group show that microvesicles shed by mesenchymal stem cells can revert the cystine accumulation in cystinotic fibroblasts. The work sponsored by the CRN may turn out to be extremely important in this respect, because the -LKG isoform may be included in microvesicles and could mediated this effect. If confirmed, this opens very interesting new research strategies to treat cystinosis.

After a period of difficulties related to the generation of stably transfected cell lines that express -LKG mutant proteins, the work is now proceeding rapidly. We remain extremely grateful to the CRN support and hope to generate data that will improve the treatment of this disease.

Anna Taranta, PhD and Francesco Emma, MD